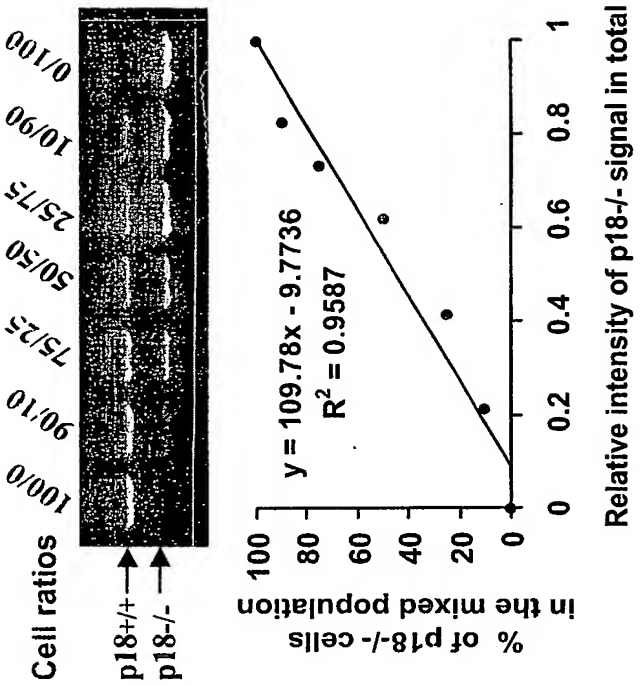


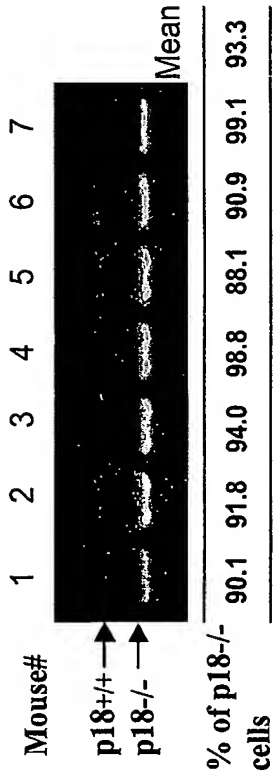


Fig. 1

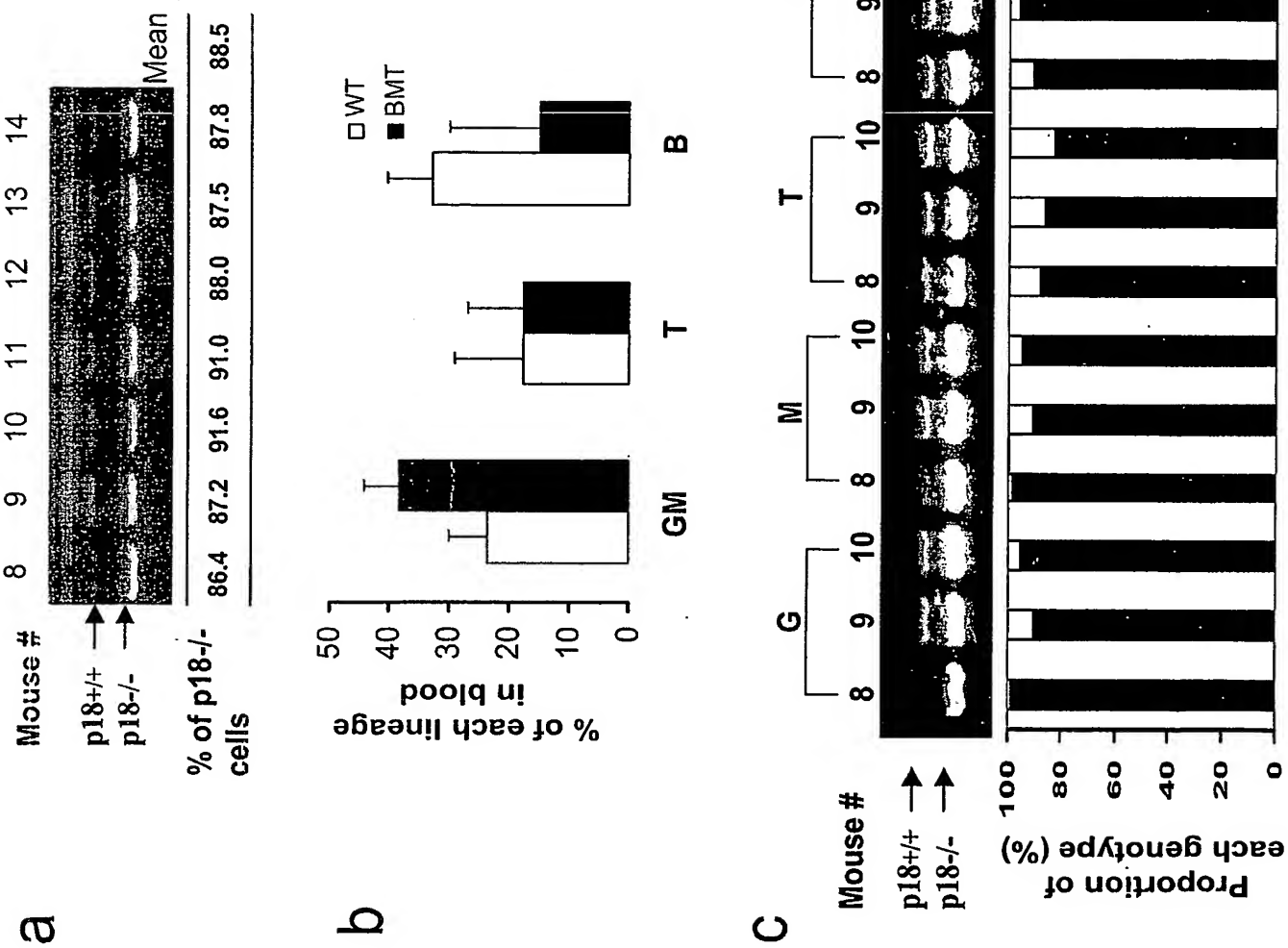
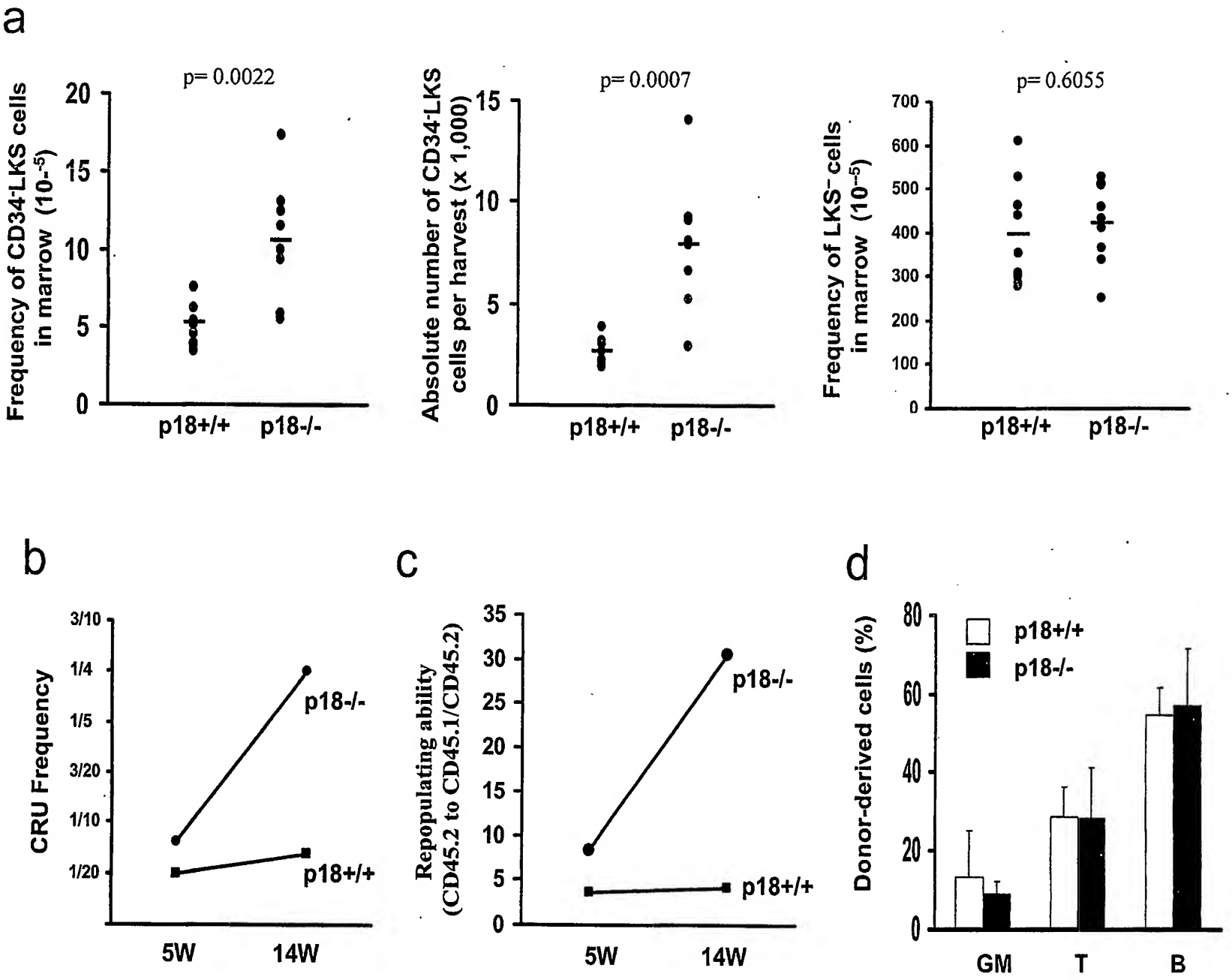

a
Mouse #
8 9 10 11 12 13 14 Mean
p18+/+
p18-/-
% of p18-/- cells
86.4 87.2 91.6 91.0 88.0 87.5 87.8 88.5
b
WT
BMT
% of each lineage in blood
50 40 30 20 10 0
GM T B
c
G M T B
Mouse #
8 9 10 8 9 10 8 9 10 8 9 10
p18+/+
p18-/-
Proportion of each genotype (%)
100 80 60 40 20 0
p18+/+
p18-/-

Fig. 2

Fig. 3


a
p= 0.0022
Frequency of CD34-LKS cells in marrow (10-5)
20
15
10
5
0
p18+/+
p18-/-
p= 0.0007
Absolute number of CD34-LKS cells per harvest (x 1,000)
15
10
5
0
p18+/+
p18-/-
p= 0.6055
Frequency of LKS- cells in marrow (10-5)
700
600
500
400
300
200
100
0
p18+/+
p18-/-
b
CRU Frequency
3/10
1/4
1/5
3/20
1/10
1/20
p18-/-
p18+/+
5W
14W
c
Repopulating ability (CD45.2 to CD45.1/CD45.2)
35
30
25
20
15
10
5
0
p18-/-
p18+/+
5W
14W
d
Donor-derived cells (%)
80
60
40
20
0
p18+/+
p18-/-
GM
T
B

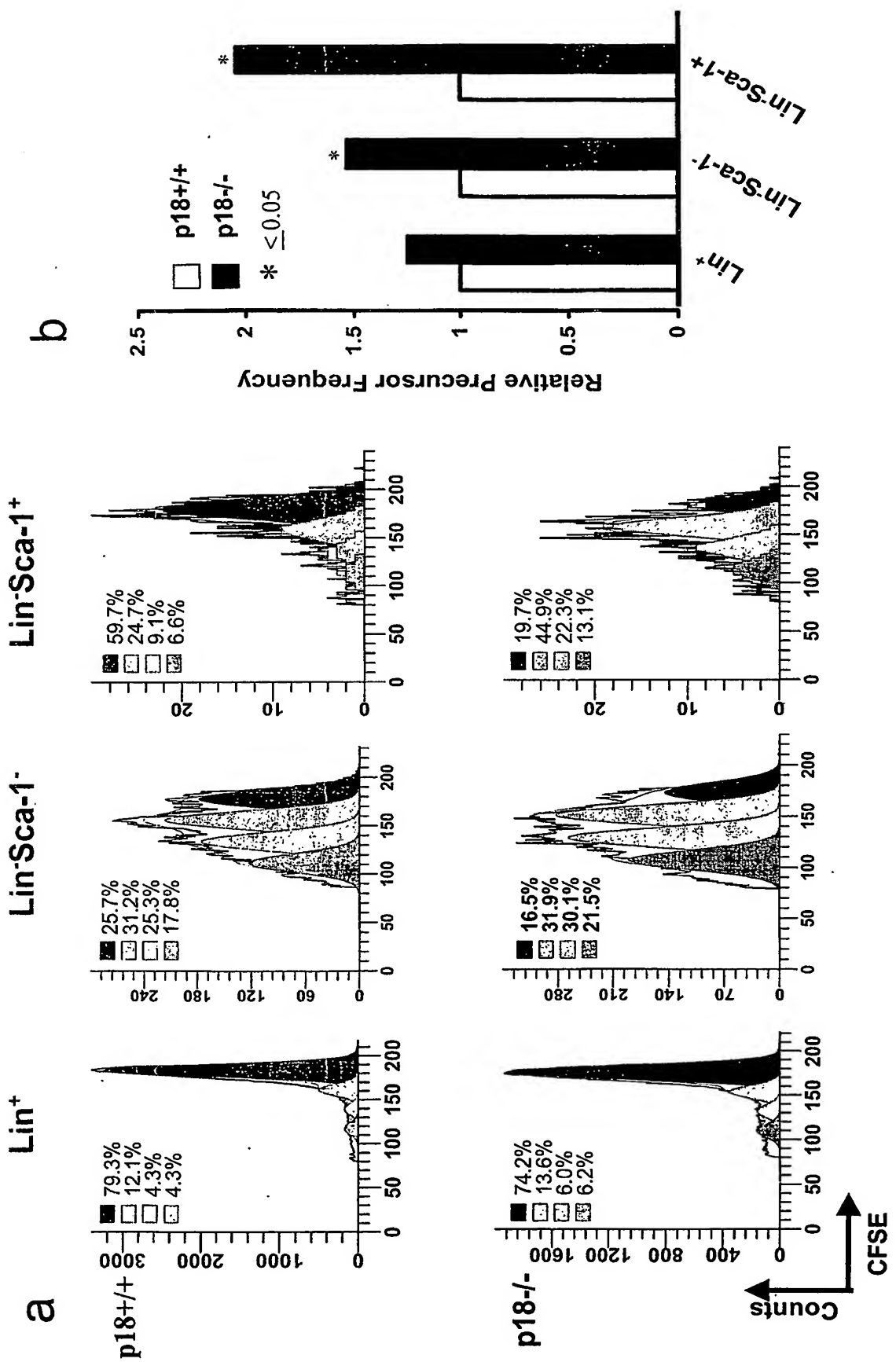
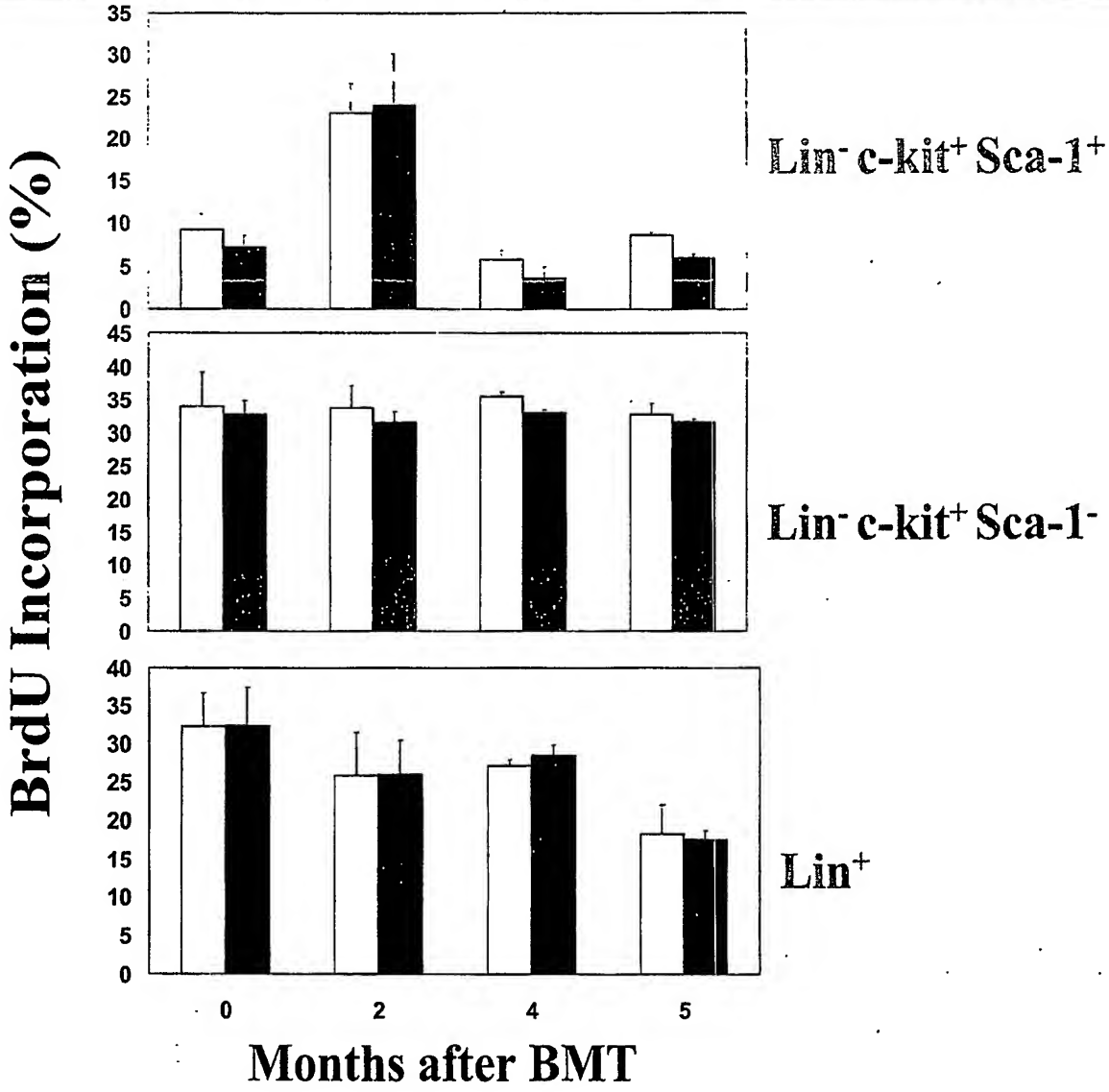

a
Lin+
Lin-Sca-1-
Lin-Sca-1+
p18+/+
p18-/-
79.3%
12.1%
4.3%
4.3%
25.7%
31.2%
25.3%
17.8%
59.7%
24.7%
9.1%
6.6%
74.2%
13.6%
6.0%
6.2%
16.5%
31.9%
30.1%
21.5%
19.7%
44.9%
22.3%
13.1%
Counts
CFSE
b
Relative Precursor Frequency
p18+/+
p18-/-
* ≤0.05
Lin+
Lin-Sca-1-
Lin-Sca-1+

Fig. 4

Fig. 5

There is no increase of BrdU incorporation in p18-/- hematopoietic cell subsets from non-transplanted (month 0) or transplanted (month 2, 4, 5) animals



(n=4-5 mice/each genotype/each time point)

Fig. 6

A similar proliferative rate between p18+/+ and p18-/- genotypes during 3-day culture after single LKS cells from non-transplanted mice were plated

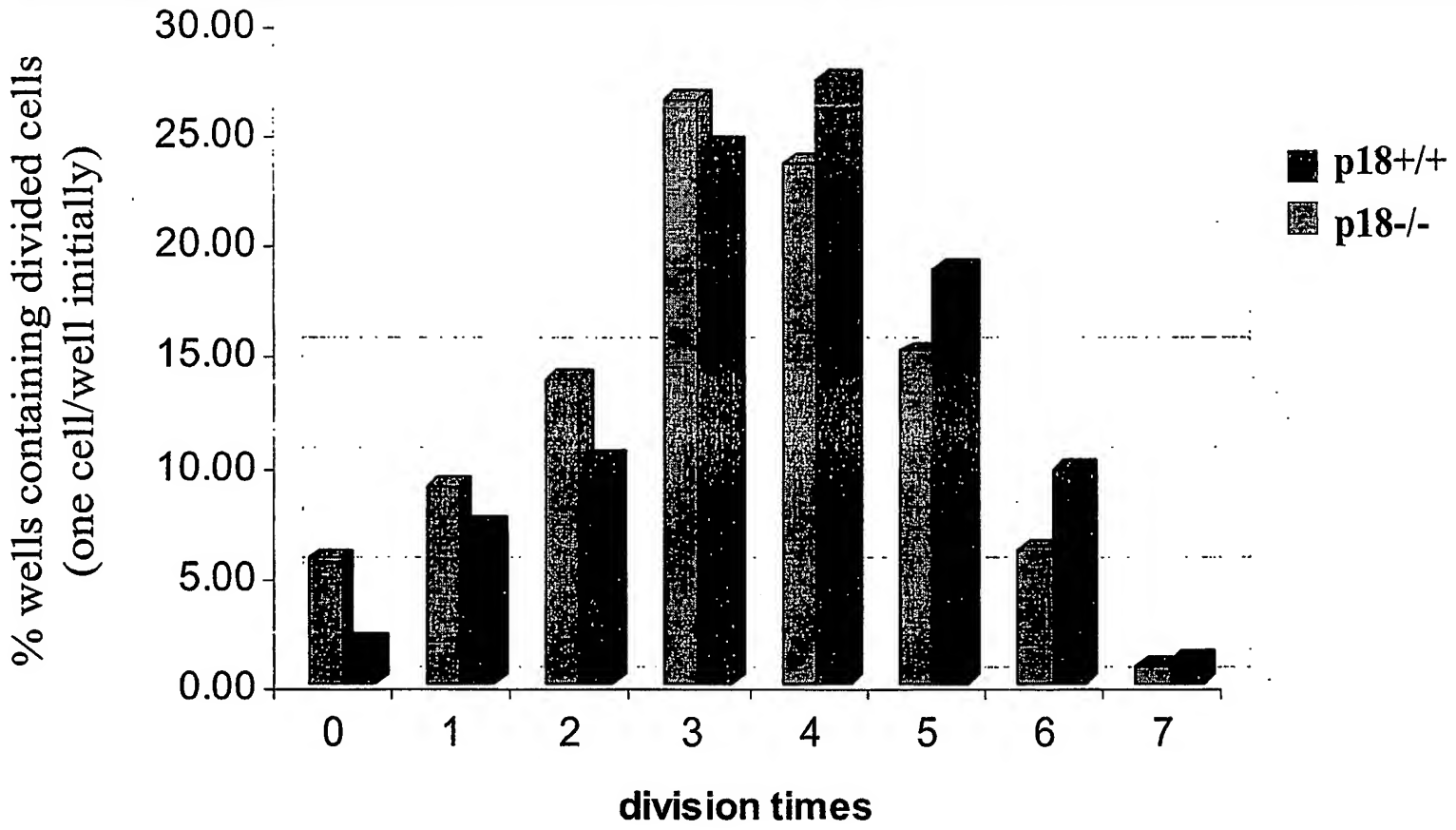


Fig. 7

A similar proliferative rate between p18+/+ and p18-/- genotypes during 3-day culture after single CD34-LKS cells from non-transplanted mice were plated

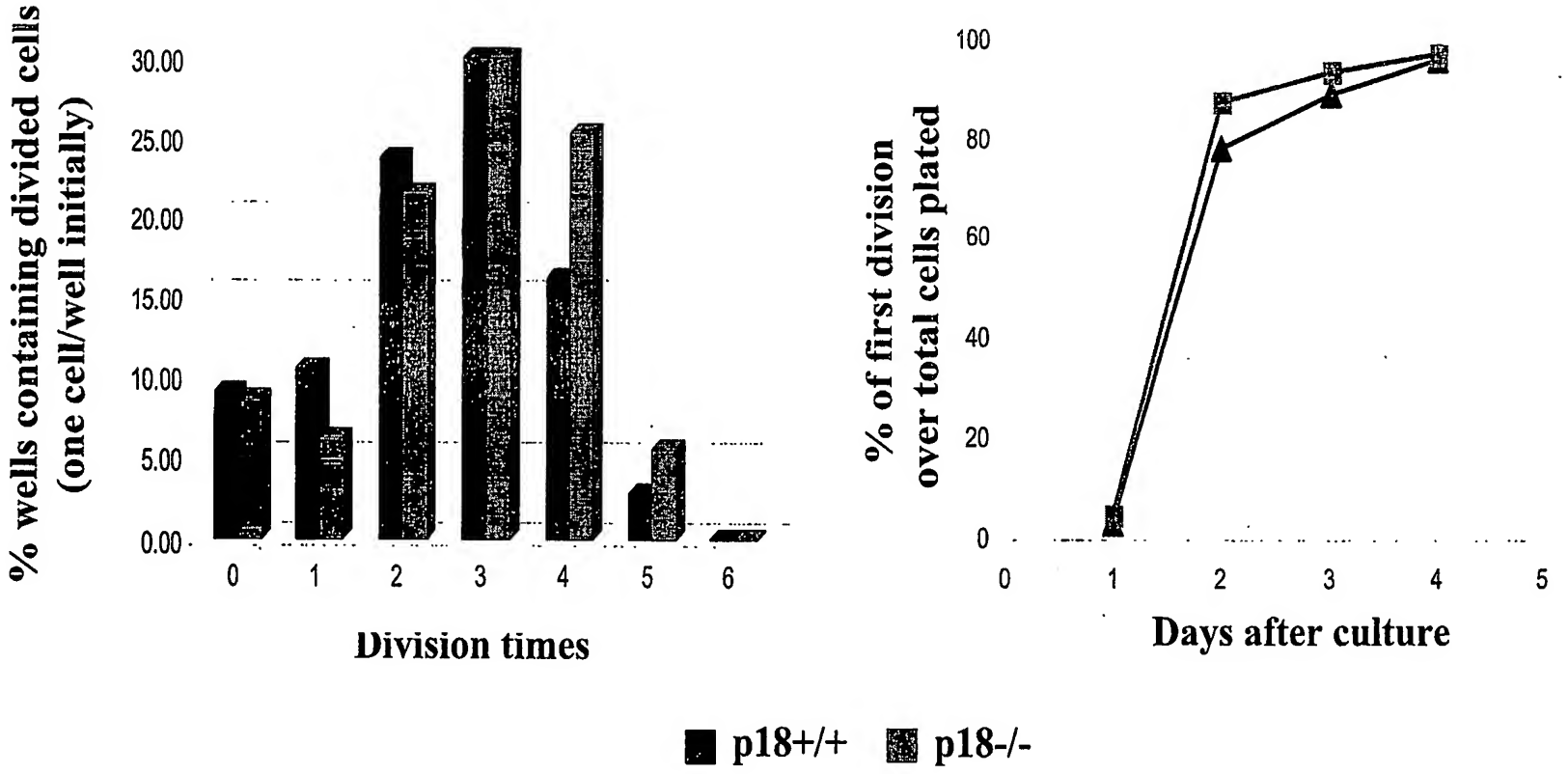


Fig. 8

A similar proliferative rate between p18+/+ and p18-/- genotypes during 3-day culture after single CD34-LKS cells from transplanted mice (2 months post transplant) were plated

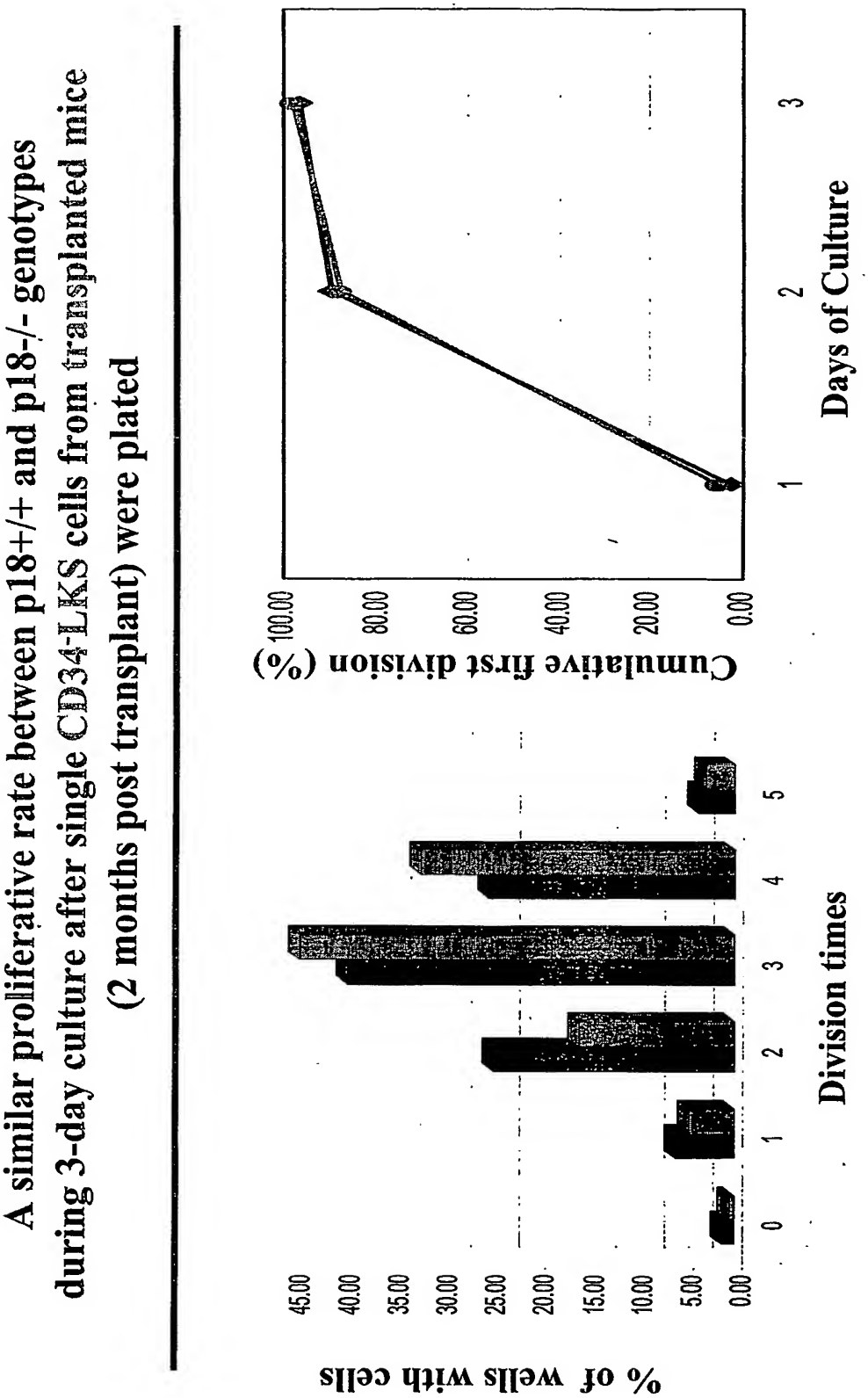
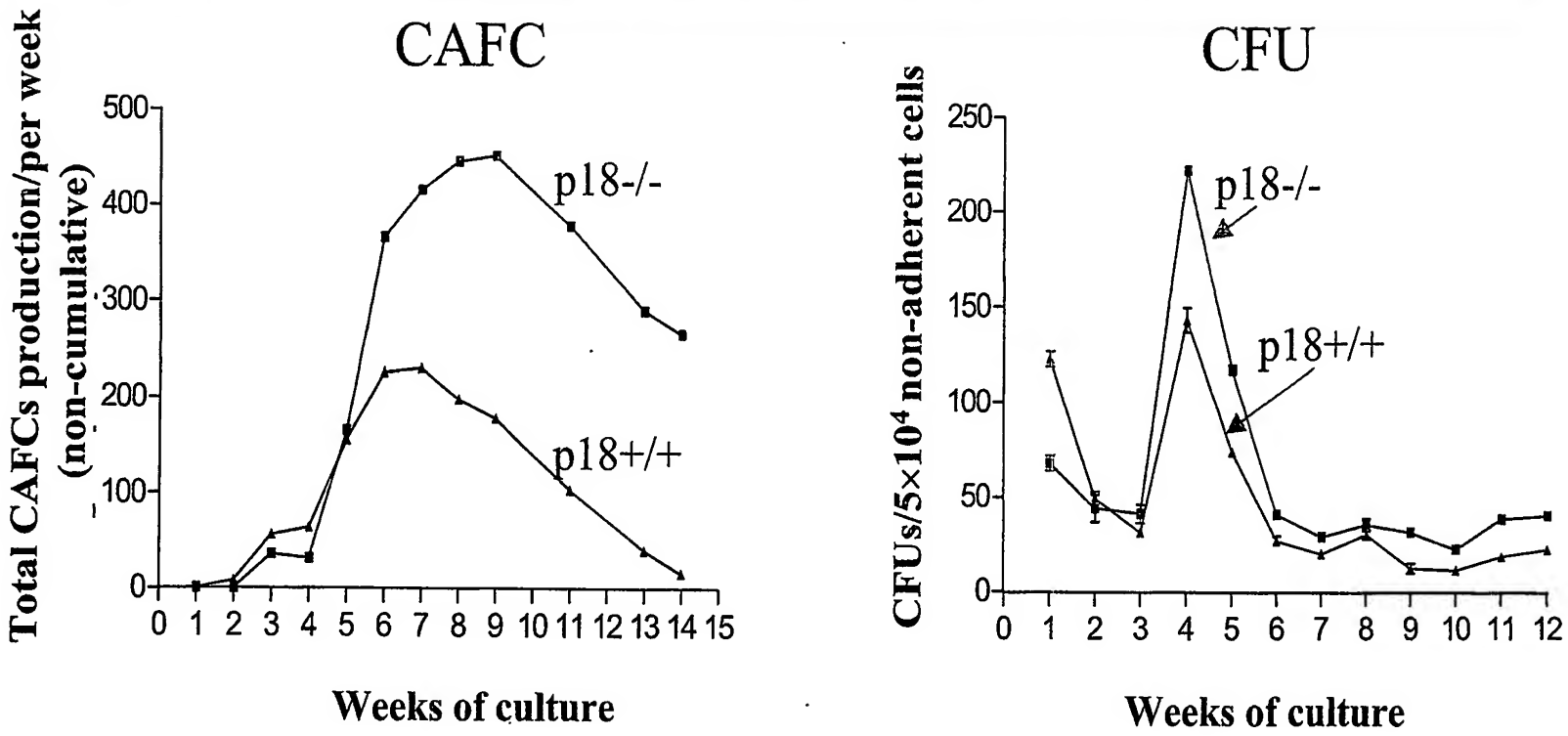


Fig. 9

Selective expansion of CAFC but not CFU during the long-term culture



Bone marrow cells from each mouse were cultured in two T75 flasks with the Dexter long-term culture medium, CAFCs were counted under microscope. Non-adherent cells were collected every week and used for CFC assay. Data were summarized from samples of 4 flasks from two mice in each group.

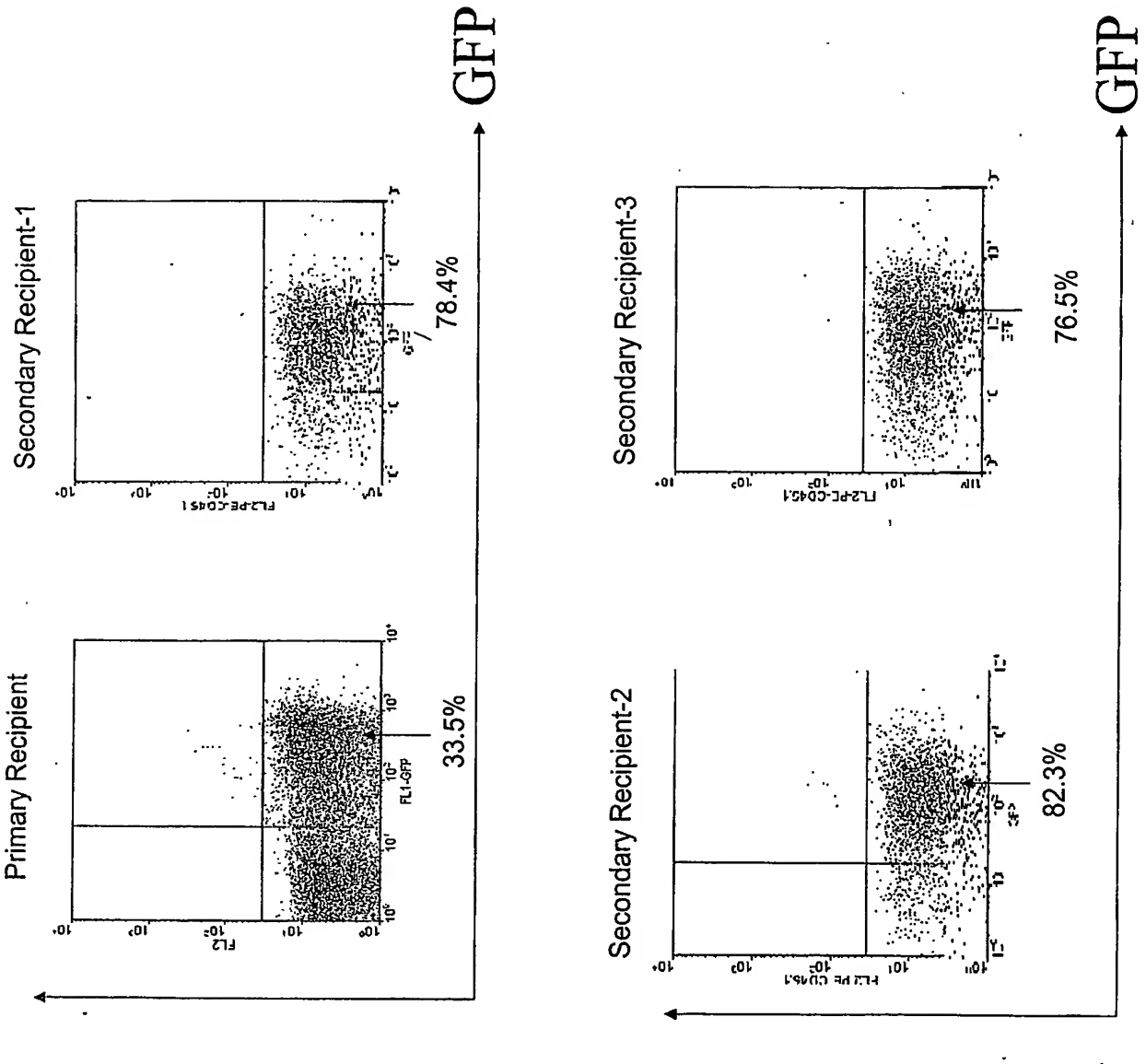

GFP
Primary Recipient
33.5%
Secondary Recipient-1
78.4%
Secondary Recipient-2
82.3%
Secondary Recipient-3
76.5%

Fig. 10

Fig. 11

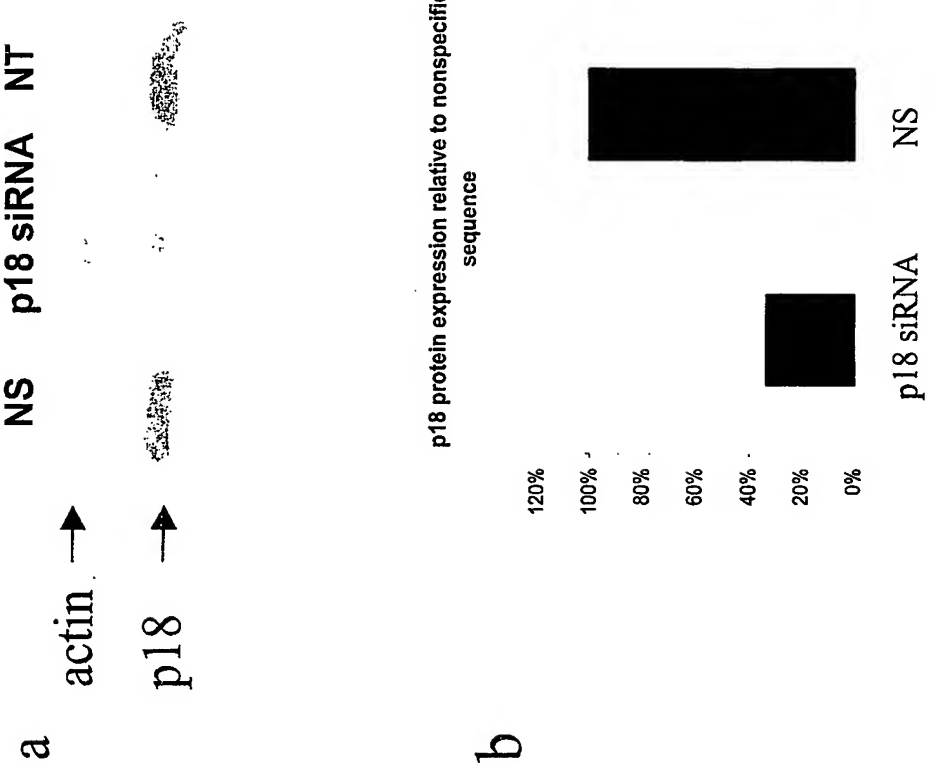

a
NS
p18 siRNA
NT
actin
p18
b
p18 protein expression relative to nonspecific sequence
120%
100%
80%
60%
40%
20%
0%
p18 siRNA
NS

Fig. 12

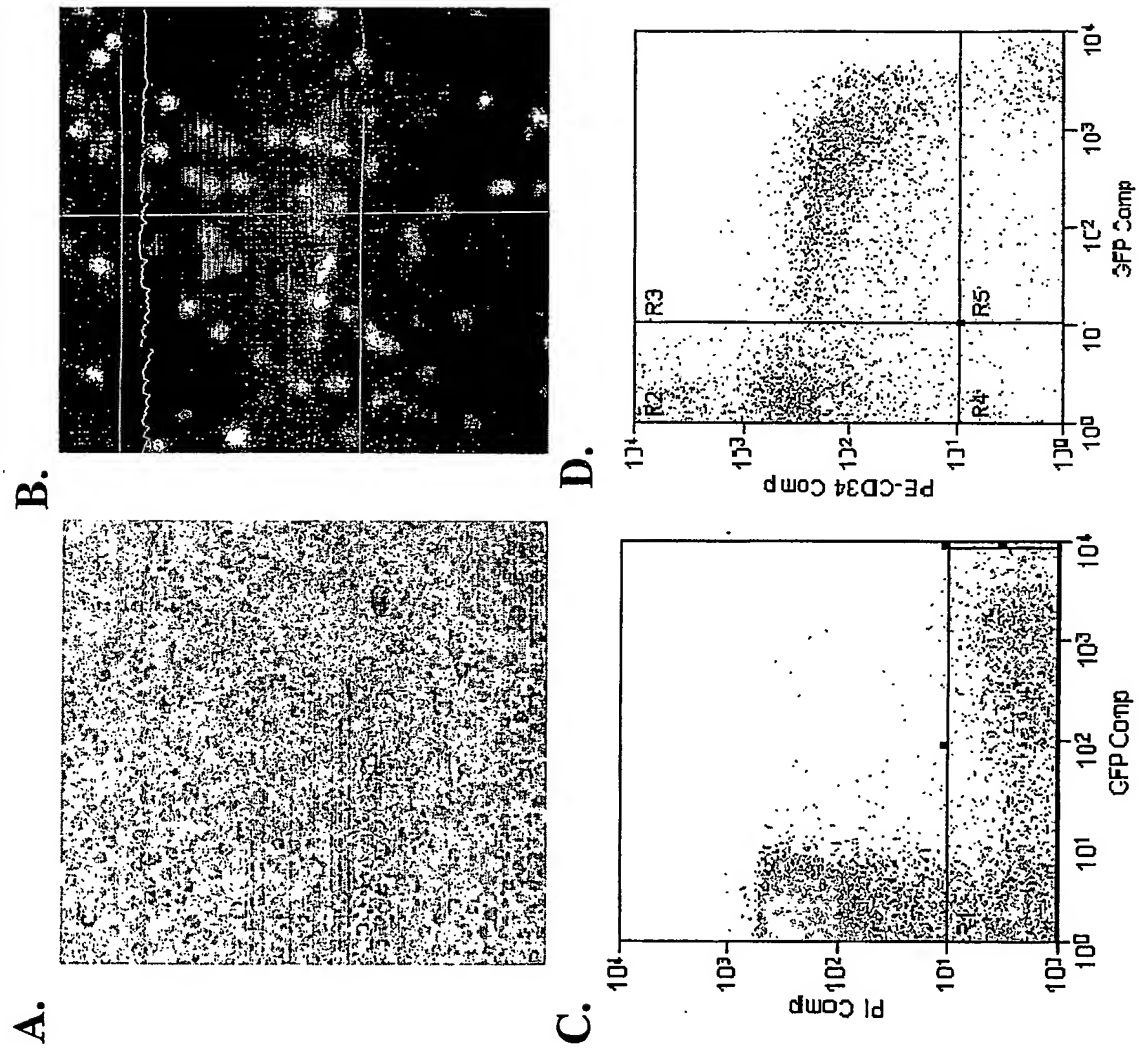


Fig. 13

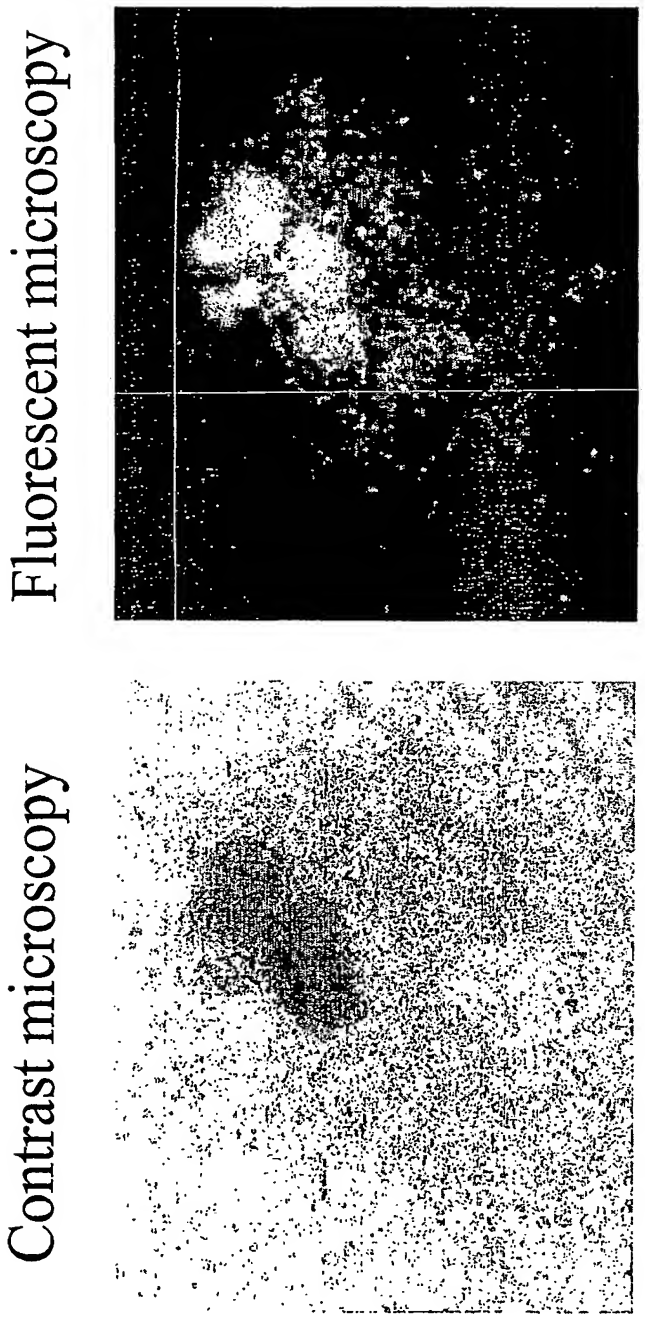


Fig. 14

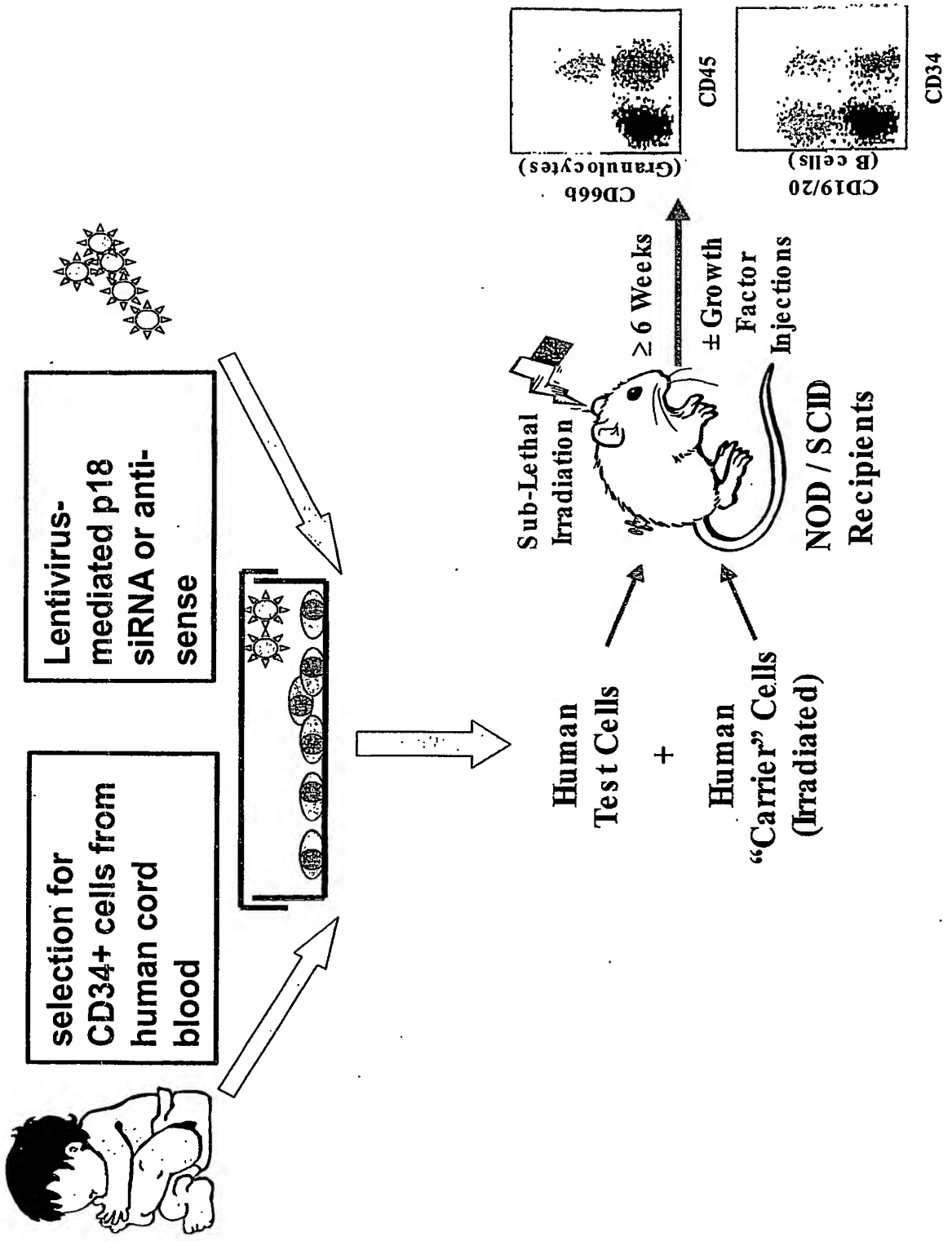
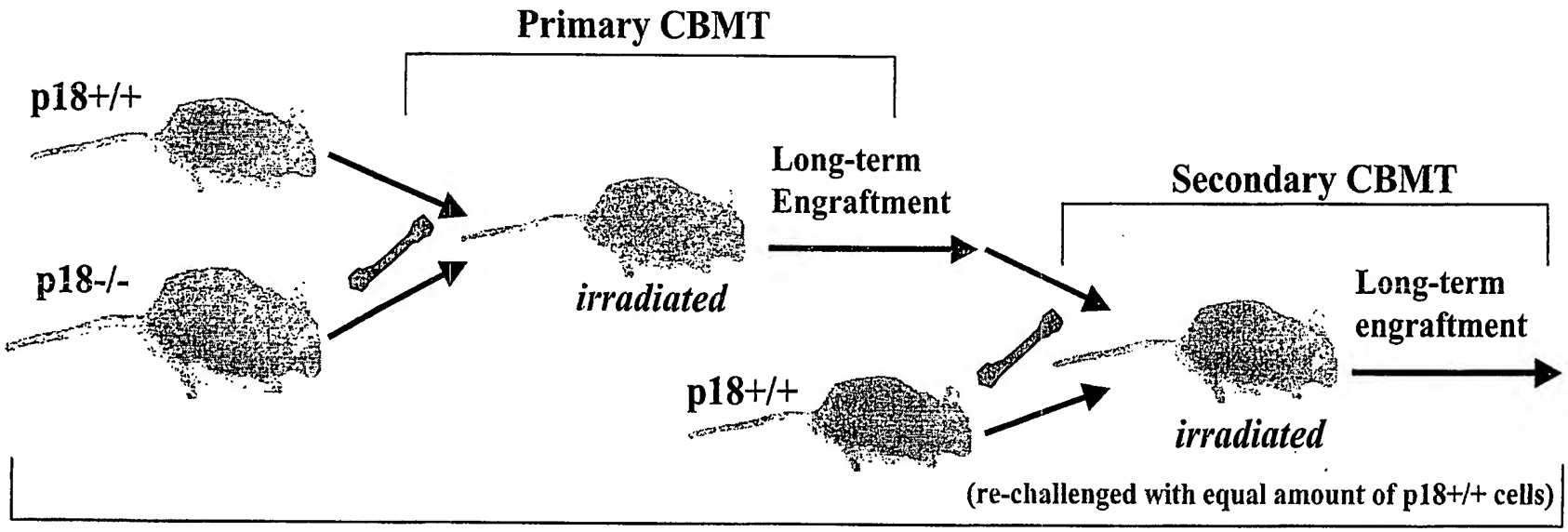

selection for CD34+ cells from human cord blood
Lentivirus-mediated p18 siRNA or anti-sense
Human Test Cells
+
Human "Carrier" Cells (Irradiated)
Sub-Lethal Irradiation
NOD / SCID Recipients
≥ 6 Weeks
± Growth Factor Injections
CD66b (Granulocytes)
CD45
CD19/20 (B cells)
CD34

Fig. 15

Fig 16

Competitive and Serial Bone Marrow Transplantation



1. Semi-quantitative PCR analysis for ratios of the two competitive cell populations in bulk cells or different lineages based on relative intensities of the two genotypic bands on the gels;
2. Single cell or colony PCR for the absolute representation of each genotype in the stem or progenitor compartment based on the appearance of a distinct band on the gel.

Fig 17

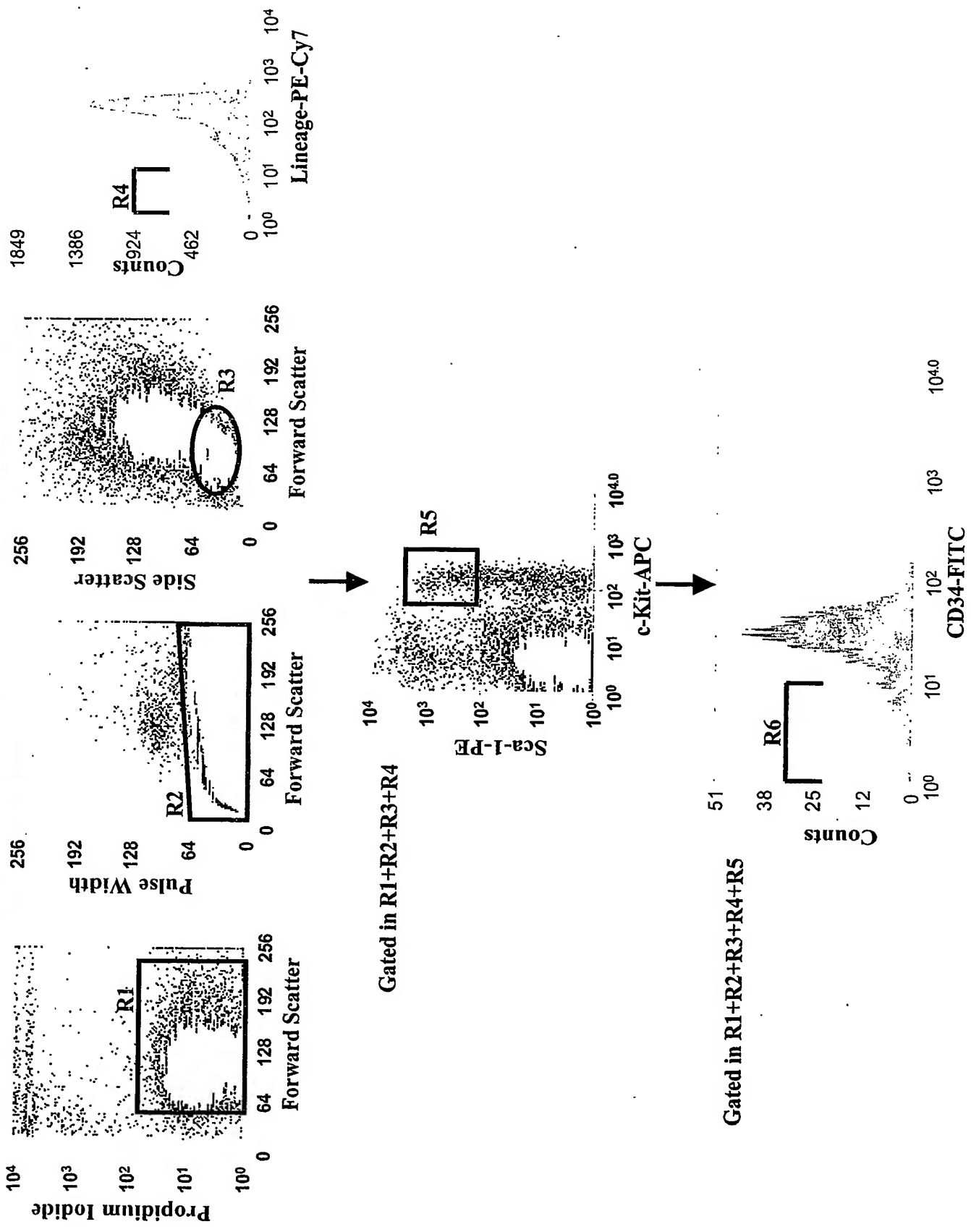
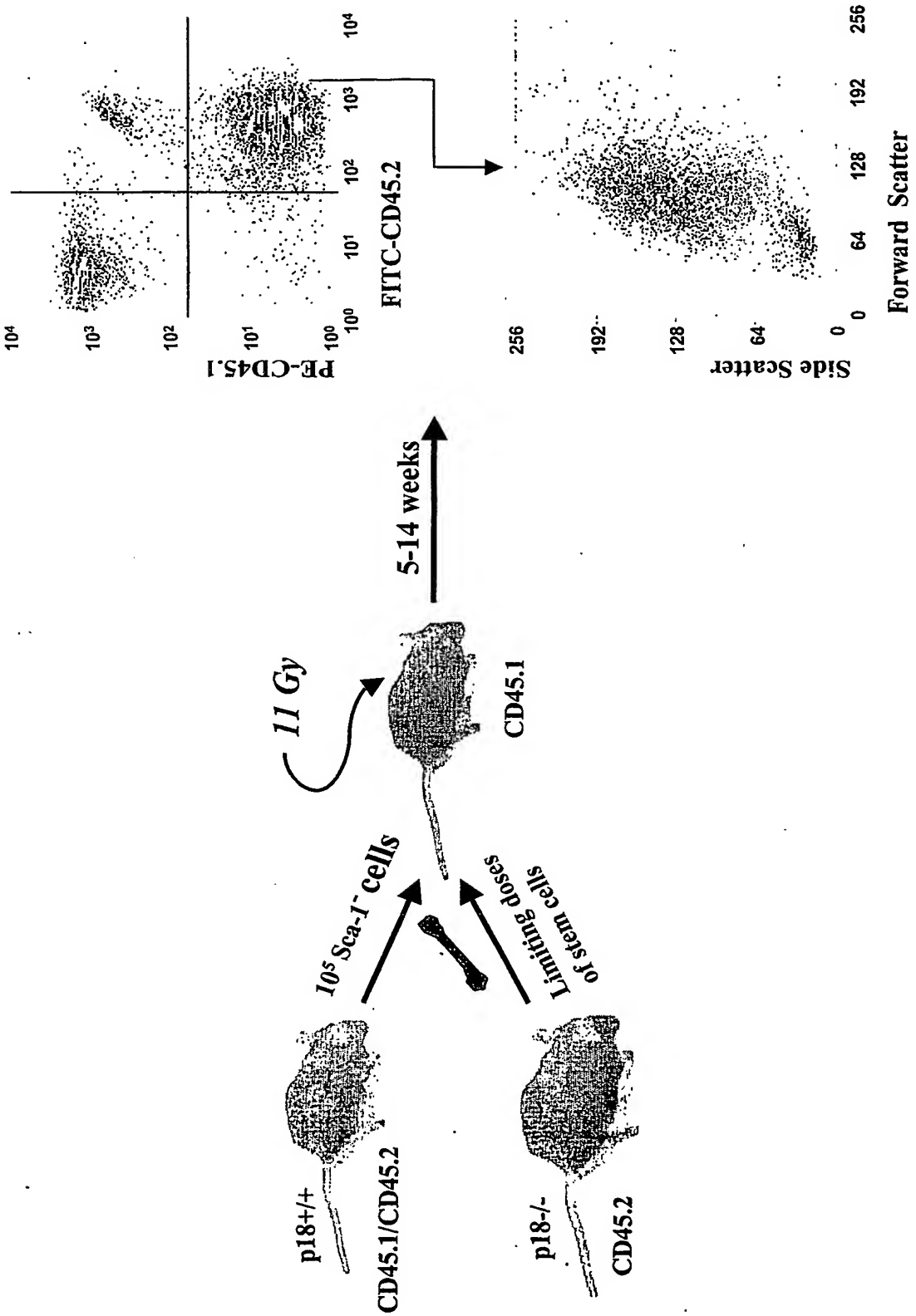

Sorting strategies for CD34-LKS cells
Propidium Iodide
Forward Scatter
R1
Pulse Width
Forward Scatter
R2
Side Scatter
Forward Scatter
R3
Counts
Lineage-PE-Cy7
R4
Gated in R1+R2+R3+R4
Sca-1-PE
c-Kit-APC
R5
Gated in R1+R2+R3+R4+R5
Counts
CD34-FITC
R6

Fig 18


Assay for the Competitive Repopulating Unit (CRU)
p18+/+
CD45.1/CD45.2
p18-/-
CD45.2
10^5 Sca-1^- cells
Limiting doses of stem cells
11 Gy
CD45.1
5-14 weeks
PE-CD45.1
FITC-CD45.2
Side Scatter
Forward Scatter